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SRF is essential for mesodermal cell migration during elongation of the embryonic body axis

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ARTICLE INFO

Article history:
Received 24 September 2013
Received in revised form
1 July 2014
Accepted 3 July 2014
Available online 11 July 2014

Keywords:
Serum Response Factor (SRF)
Brachyury
Mouse embryo
Body axis elongation
Migration
Epithelial–mesenchymal transition

ABSTRACT

Mesoderm formation in the mouse embryo initiates around E6.5 at the primitive streak and continues until the end of axis extension at E12.5. It requires the process of epithelial-to-mesenchymal transition (EMT), wherein cells detach from the epithelium, adopt mesenchymal cell morphology, and gain competence to migrate. It was shown previously that, prior to mesoderm formation, the transcription factor SRF (Serum Response Factor) is essential for the formation of the primitive streak. To elucidate the role of murine *Srf* in mesoderm formation during axis extension we conditionally inactivated *Srf* in nascent mesoderm using the *T(s)::Cre* driver mouse. Defects in mutant embryos became apparent at E8.75 in the heart and in the allantois. From E9.0 onwards body axis elongation was arrested. Using genome-wide expression analysis, combined with SRF occupancy data from ChIP-seq analysis, we identified a set of direct SRF target genes acting in posterior nascent mesoderm which are enriched for transcripts associated with migratory function. We further show that cell migration is impaired in *Srf* mutant embryos. Thus, the primary role for SRF in the nascent mesoderm during elongation of the embryonic body axis is the activation of a migratory program, which is a prerequisite for axis extension.

1. Introduction

During vertebrate development the embryonic body grows in an anterior to posterior direction. Elongation of the antero-posterior (A–P) axis depends on the progressive addition of new tissue at the posterior end of the embryo, which is generated by a transient structure called the primitive streak (ps). In mouse embryos, the ps forms at embryonic day (E) 6.5 and is replaced by the tail bud at mid-somite stages (E9.25–E9.5, ⩾22 somites) (for review, see Beddington, 1983).

Cells located in the ps differentiate into mesendodermal tissue, marked by the expression of genes such as *Brachyury* (*T*) (Herrmann, 1991). These cells undergo an epithelial-to-mesenchymal transition (EMT), allowing them to become motile, migrate away from the ps, and intercalate between the definitive endoderm and ectoderm (reviewed in Baum et al., 2008). Epithelial epiblast cells provide a constant source of mesodermal cells. At E7.5 a complete layer of mesoderm has formed between the ectoderm and endoderm, but EMT continues until axis elongation ceases between E12.5 and E13.5 (Cunningham et al., 2011).

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http://dx.doi.org/10.1016/j.mod.2014.07.001
0925-4773/

EMT has been shown to be a central process during various stages of embryonic development including gastrulation, neural crest formation, and heart morphogenesis (Lim and Thiery, 2012). Moreover, the essential role of EMT in tumor metastasis has been well studied. EMT is a multistep process that comprises the degradation of cell–cell contact proteins (e.g., E-cadherin), the breakdown of the basement membrane, and the regulation of cytoskeletal reorganization (Nakaya and Sheng, 2008; Levayer and Lecuit, 2008). As a consequence, mesenchymal cells lose contact with their neighboring cells, which is a prerequisite for migratory capacity, and they adopt a more extended and elongated shape.

The molecular basis that underlies EMT during mouse gastrulation is only partly understood. Genetic studies in mice have demonstrated that FGF induces downregulation of E-cadherin in ps cells, both at the transcriptional and post-transcriptional levels (Ciruna and Rossant, 2001; Zohn et al., 2006), thereby inducing the delamination of cells from the epithelium. In addition to FGF, several other extracellular signaling molecules contribute to EMT in generating mesodermal cells, including members of the Wnt and TGFβ families (Ben-Haim et al., 2006; Kemler et al., 2004). It has also been shown that Wnt signaling is required to specify a pool of multipotent stem cells in the caudal region of vertebrate embryos (Martin and Kimelman, 2012), and that these axial stem cells are required for vertebrate axis extension (for review see Wilson et al., 2009). The molecular characterization of these stem cells is pending, but it is already established that *Sox* genes are involved in their function (Yoshida et al., 2014).

The gene encoding the transcription factor Serum Response Factor (SRF) is expressed in the ps and newly formed mesoderm in several vertebrates at early stages of gastrulation (Barron et al., 2005; Croissant et al., 1996; Mohun et al., 1991), but its role during mesoderm development and axis elongation has not received careful investigation. SRF is a homodimeric MADS-box-containing transcription factor, which binds a 10 bp sequence known as the CArG box (Norman et al., 1988; Shore and Sharrocks, 1995). More than 170 *bona fide* SRF target genes have been identified (reviewed in Miano, 2008). These mainly comprise genes involved in muscle cell differentiation, as well as the regulation of cell growth, cell survival, and cell motility (Johansen and Prywes, 1995; Miano, 2003).

Srf$^{-/-}$ mouse embryos show severe gastrulation defects, including lack of the ps, and die at mid-gestation (Arsenian et al., 1998). *Srf* mutants lack all mesodermal tissue and begin to be resorbed from stage E8.5 onwards. While these data identified SRF as a regulator of mesoderm induction, they did not provide insight into the role that SRF plays once the ps has formed. Thus, in order to study the role of SRF during axis elongation, we conditionally deleted *Srf* in murine ps cells using Cre recombinase driven by the *Brachyury* (*T*) promoter region responsible for *T* expression in the ps (*T*(*s*)::*Cre*) (Feller et al., 2008). The resulting mutant embryos displayed, amongst other defects, severe axis truncation – indicating that SRF is essential for axis elongation. We present both molecular and functional data to demonstrate that SRF is required for mesodermal cell migration during axis elongation. This identifies SRF as a main player in the EMT process during gastrulation and the subsequent mesodermal cell movements that are essential for axis elongation.

2. Results

2.1. *Conditional deletion of Srf in Brachyury-expressing cells causes an arrest of axis elongation*

In order to investigate the role of SRF during trunk development, we first analyzed its expression pattern in mid-gestational embryos. Whole mount *in situ* hybridization of E9.5 mouse embryos showed domains of increased *Srf* expression in the forebrain, the branchial arches, the intermediate mesoderm and in the paraxial mesoderm and caudal end (Fig. 1A). Expression of *Srf* in nascent mesodermal tissue (such as the paraxial mesoderm) suggested a possible role in mesoderm development during axis elongation. To analyze the function of SRF in nascent mesoderm we generated mouse embryos lacking *Srf* in that tissue using the Cre/loxP system. *Brachyury* (*T*) is one of the earliest markers of mesoderm during development (Herrmann, 1991), thus, we crossed the *Srf*$^{flex1/flex1}$ mouse line carrying a loxP flanked exon one of *Srf* (Wiebel et al., 2002) with the *T*(*s*)::*Cre* (C57Bl/6J$^{Tg(T\text{-}cre)1Gos}$) line expressing Cre recombinase under control of the T_{streak} promoter of *T*, comprised of the 600 bp upstream of the ATG codon (Feller et al., 2008). This results in a reduction of *Srf* in anterior mesoderm of E8.5 embryos and a complete loss *Srf* expression in posterior mesodermal tissue (Fig. 2B).

The morphology of *Srf*$^{flex1/flex1}$; *T*(*s*)::*Cre* embryos was indistinguishable from wild type embryos until E8.5, but at E8.75 embryos showed malformations of the heart, the allantois, and had an overall smaller appearance (Fig. 1C and D). At E9.5, axis elongation in the mutants had been fully arrested, demonstrating an essential function for SRF in this process. At E10.5 no viable *Srf*$^{flex1/flex1}$; *T*(*s*)::*Cre* embryos were observed. The role of SRF in heart development has already been described (Parlakian et al., 2004), and abnormalities of the heart and allantois, resulting in defective nutrient and gas exchange, are the likely cause of death in the current model. In addition, this phenotype is in full agreement with previously observed impairment of mesoderm formation upon constitutive *Srf* deletion (Arsenian et al., 1998).

2.2. *Mesenchymal character is not impaired in cells of the Srf*$^{flex1/flex1}$*; T(s)::Cre embryo caudal end*

The arrest of axis elongation observed here could be caused by the loss of mesoderm cells, therefore differing from *Wnt3a* or *Brachyury* mutant embryos where mesoderm induction and formation is impaired (Takada et al., 1994; Wilkinson et al., 1990). In order to investigate this possibility we tested for the presence of BRACHYURY-positive cells in *Srf* mutant embryos by immunohistochemistry. *Srf* mutants have a significant number of BRACHYURY positive cells, indicating that mesoderm is indeed formed upon loss of SRF in cells progressing through the ps (Fig. 2A). An early hallmark of mesoderm formation is the initiation of EMT in the ps accompanied by loss of the cell surface marker E-cadherin. In *Srf* mutants EMT seems to be initiated, as E-cadherin is present